PROTEIN BINDING OF DIMETRIDAZOLE IN *SALMONELLA TYPHIMURIUM*: A MODEL FOR THE STUDY OF COVALENT PROTEIN ADDUCT FORMATION.

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Dimetridazole (**DMZ**) is an economically important veterinary antimicrobial which has also been shown to be a weak carcinogen. The mechanism of action has been demonstrated to involve nitro-reduction and binding of reactive metabolites to macro-molecules. Regulatory agencies have expressed concern because a small percentage of the **DMZ** is retained as covalently bound protein adducts. These adducts are of interest due to the potential for release of toxic compounds after their cleavage from protein by the enzyme ß-lyase.

The protein binding of metabolites of 2-[^{14}C]-**DMZ** in whole cells and soluble protein was studied in *S. typhimurium* strains TA98NR, TA98, and YG1021 which respectively contain low, moderate and high levels of nitro-reductase. Protein binding was 16 and 44 times greater in TA98 and YG1021 than in TA98NR. There was also good correlation between the extent of metabolism and mutagenicity of **DMZ**, and levels of nitroreductase in the different strains. The release of radiolabel from protein was enhanced by the presence of a cell-free bacterial extract. The release of label was inhibited by (aminooxy)-acetic acid, a ß-lyase inhibitor. Studies are on-going to identify the released amino-acid-**DMZ** adducts.

LISTERIA MONOCYTOGENES INFECTION DECREASES CYTOCHROME P-450-MEDIATED METABOLISM BY A PRE-TRANSLATIONAL MECHANISM.

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Listeria monocytogenes is a hemolytic gram positive bacteria. In microsomes isolated from mice 48 hours after the administration of $1x10^6$ CFU of Listeria monocytogenes type 4b (strain 15U), cytochrome P-450 content, ethoxyresorufin-O-dealkylase (EROD), benzyloxyresorufin-O-dealkylase (BROD), and pentoxyresorufin-O-dealkylase (PROD) activities were suppressed by 40-60%. Cytochrome P-450IAI mRNA was significantly reduced in β-napthaflavone - induced mice but there was no change in total mRNA as determined using a poly(T) probe. A non-hemolytic avirulent strain of listeria (M3D) had no effect on cytochrome P-450 content or on EROD, BROD, or PROD activities.
In a culture system of liver cells (hepatocytes+ non-parenchymal cells), the 15U strain suppressed cytochrome P-450 content as well as EROD activity in hepatocytes after 24 hour incubations; in the absence of any change in viability. The 15U strain of listeria had no effect on preparations of isolated hepatocytes, consistent with an indirect mechanism involving the release of a factor from kupffer cells. The M3D strain had no effect in liver cell cultures.
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PRESENCE OF COUMARIN AND TESTOSTERONE HYDROXYLASES IN REINDEER LIVER.

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We have characterized the inducibility and species differences of P450Coh which catalyzes the 7-hydroxylation of coumarin. This cytochrome has recently been shown to be closely related to P450-15α which catalyzes testosterone hydroxylation at the 15α-position. These two isozymes have been nominated as Cyt2A4 and 2A5 in murine liver. In the present study we investigated the level of these two enzymes in reindeer which is a specific species for Northern countries.
The activities of coumarin 7-hydroxylase (COH) and various testosterone hydroxylases were studied in 10 adult and 10 young reindeer liver samples. The relative activity ratios were 10:1:2 for COH and 10:100:200 for 15α in human, reindeer and mouse livers, respectively.The activity of COH in young animals were about 40% lower than in adults but the testosterone 15α-hydroxylase activity in young animals reached already the adult level.The testosterone 6β- hydroxylase was significantly lower in youngsters.

METABOLISM AND DISPOSITION OF CPT-11, AN ANTINEOPLASTIC AGENT, IN RATS. R. Atsumi, W. Suzuki, N. Yoshida and H.Hakusui Research Institute, Daiichi Pharmaceutical Co.,Ltd., Tokyo, Japan.

CPT-11 is a new camptothecin derivative which is currently being clinically evaluated for the treatment of cancer. To determine metabolic fate of this compound in vivo, carbon-14 labeled CPT-11 was intravenously administered to rats.

Plasma radioactivity was decayed in multi-exponential manner with the terminal half life of 8.2 hr. The autoradiogram showed that radioactivity was rapidly distributed thoughout the body except central nervous system. TLC analysis on the liver, kidney, lung and pancreas homogenate showed that unchanged drug is dominant in these organs.

Ninety-nine percent of radioactivity was recovered in feces and urine(77% and 22%, respectively) within 72 hrs after administration. In bile-duct canulated rats, 62% of radioactivity was recovered in rat bile. Nine percent of radioactivity was recovered in feces from bile-duct canulated rats, suggesting that CPT-11 was partly secreted from the intestine.

An unknown metabolite detected in rat bile was isolated and purified by HPLC. The metabolite was identified as SN-38 glucuronide by its NMR, mass spectra and β-glucuronidase hydrolysis.

THE PHARMACOKINETICS OF ALMOKALANT, A NOVEL CLASS III ANTIARRHYTHMIC AGENT, AND ITS FOUR STEREOISOMERS IN THE DOG

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Almokalant is a new class III antiarrhythmic agent which is active after both intravenous and oral administration. The compound has two chiral centers and consequently appears as four stereoisomers. In most of the pharmacokinetic studies the stereoisomeric mixture (M) has been used. This presentation includes pharmacokinetic data of the isomeric mixture and of each stereoisomer in the dog.

An oral dose of almokalant (M) is rapidly absorbed and C_{max} is reached within one hour post dose. A rapid distribution is followed by an elimination phase with a $t_{1/2}$ of 6 hours which is comparable to the terminal half life after the intravenous dose. The total body plasma clearence (CL) of almokalant is 33 $ml \cdot min^{-1} \cdot kg^{-1}$. The ratio between the concentration in plasma and blood is 1. The volume of distribution at steady state (V_{SS}) is estimated to 4 $L \cdot kg^{-1}$. Renal excretion is of major importance for the elimination, >80 % of the radiolabelled dose being recovered in urine where both unchanged drug and metabolites have been identified. The systemic bioavailability (F) of orally administered almokalant is about 60 %.

The substance exhibits linear pharmacokinetics within the dose interval 2–40 µmol/kg. No accumulation is observed after repeated adminstration once a day and there is no difference in pharmacokinetics between sexes.

Studies of the individual enantiomers indicate only minor differences between their pharmacokinetic properties. The R–enantiomers have slight higher clearences than the corresponding S–forms.

BIOMONITORING OF OCCUPATIONAL EXPOSURE TO 4,4'-METHYLENEDIANILINE BY THE GC-MS DETERMINATION OF ADDUCTS TO HAEMOGLOBIN

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4,4'-Methylenedianiline (MDA) is an aromatic diamine widely used as a curing agent in the plastic industry. The determination of the covalent bound reaction products to haemoglobin has been investigated as a dosimeter for occupational exposure to this potential human carcinogen. Twenty-four hr following a single i.p. dosage to rats with ^{14}C-MDA (60.61 µmol/kg) 0.4% of the administered dose was adducted to the haemoglobin. Base hydrolysis liberated two adducts MDA and N-acetyl MDA which accounted for 40% of the bound radioactivity. A specific and sensitive assay procedure has been developed for quantifying both the base released protein adducts. The method utilizes solvent extraction followed by derivatization with pentafluro-proprionic anhydride and then separation and quantitation by capillary gas chromatography with electron impact selective ion monitoring mass spectrometry. Deuterium-labelled analogues of MDA and N-acetyl MDA are used as internal standards. The method has been used to determine the stability of the adducts in rat haemoglobin ($t_{\frac{1}{2}}$=10.4 days) and to establish a linear dose-response relationship in i.p. dosed rats between production of each adducts and dose of MDA (5.05-60.61 µmol/kg). The method which has a detection limit for both adducts of less than 10 pmol/g haemoglobin, is being used to monitor short term and chronic exposure in industrial workers exposed to MDA.

COMPARISON OF THE PERCUTANEOUS ABSORPTION OF [^{3}H]-FLUTICASONE 17-PROPIONATE FROM SCALP SOLUTION AND OINTMENT FORMULATIONS IN THE RAT.

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The percutaneous absorption of radioactivity was investigated in male and female Sprague-Dawley rats following a 24 hour topical application of [^{3}H]-Fluticasone 17-propionate in solution (0.0493% w/w) or ointment (0.0506% w/w) formulations (1 g formulation/kg body weight). The mean maximum plasma concentrations (Cmax) of [^{3}H]-Fluticasone 17-propionate-derived radioactivity were 2.92 ± 0.43 and 1.61 ± 0.54 ng equivalents per g plasma for the ointment and solution formulations, respectively ($p<0.01$). Time to maximum detected concentrations was 38 h (ointment) and 67 h (solution). Mean areas under the plasma concentration time-curves (AUC_{0-168}) were 271 ± 63 ng.hr/g (ointment) and 156 ± 51 ng.hr/g (solution) ($p<0.01$). When data were normalized to a dose of 500 μg equivalents/kg, the same statistically significant ($p<0.01$) differences in Cmax and AUC_{0-168} were observed. The total recovery of administered radioactivity was greater than 90% for both formulations (mean 92.7%), most of which was recovered from the site of application at 24 h. A very small proportion of the total could be accounted for by excretion into urine: less than 0.2% was recovered in the urine by 168 h. By 168 h, 6.2% (ointment) and 14.1% (solution) of the administered radioactivity was recovered in feces. By 168 h, the skin, excised from the site of application, contained 0.26% and 1.67% (ointment and solution respectively) while less than 1% of the dose remained in the carcass. Results suggest that, based on plasma data, the absorption of [^{3}H]-Fluticasone 17-propionate-derived radioactivity is greater following topical application of the ointment formulation than following topical application of the solution formulation when each is applied at a dose of 1 g/kg body weight.

INHIBITION OF HUMAN STEROID SULPHOTRANSFERASES BY DRUGS- IMPLICATIONS FOR ADVERSE DRUG REACTIONS.

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Adverse drug reactions are a common feature of modern drug therapy and these effects are costly both in medical and economic terms. We are addressing this problem by investigating the role that the inhibition of the sulphotransferases (STs), in particular the steroid STs, may play in these adverse reactions. These enzymes catalyze the sulphation reaction, an important step in the biotransformation of xenobiotic and endogenous compounds e.g: steroid hormones. We have investigated the inhibition of cytosolic dehydroepiandrosterone (DHEA) ST in a number of different human liver samples by a wide range of commonly prescribed drugs. Strong inhibition (up to 90%) was observed with ethinylestradiol, similarly with epiandrosterone and danazol. The tricyclic antidepressants amitriptyline and imipramine again strongly inhibited DHEA ST (up to 65%) indicating that this competition may provide a molecular basis for the frequent side effects exhibited by this class of drug.
The implications of this work and the relevance of the role of STs in adverse drug reactions will be discussed.

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CHLORDANE ALTERATION OF MACROPHAGE CYTOKINE PRODUCTION.

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Prenatal exposure to chlordane decreased the delayed-type hypersensitivity (DTH) response of adult BALB/c mice, however, lymphocyte reactivity was not adversely affected, suggesting an effect on macrophage (mo) function. In the present study, mo obtained from thioglycollate (TG) stimulated mice, prenatally exposed to 8 mg/kg chlordane, displayed severely depressed cytotoxicity to tumor cells after culturing for 24 or 48 hours in the presence of gamma-IFN and LPS. To examine the effects of chlordane on the levels of tumor necrosis factor (TNF) within the mo total RNA was isolated from peritoneal mo from chlordane- or vehicle-treated mice cultured for 2, 24, or 48 hours. The TNF mRNA transcript from resident mos of both vehicle and chlordane treated was minimal, however, stimulation with TG greatly enhanced TNF mRNA transcription in vehicle mos after culturing for only 2 hours. Chlordane-treated mos did not display an increase in TNF mRNA transcription until the cells were cultured for 24 hours. TNF possesses pleomorphic effects on the immunological system. Although the mechanism is not presently known, prenatal treatment with chlordane influences these properties thus altering the mo functional abilities. U.S.P.H.S. - NIH grant ES02875.

IDENTIFICATION OF THE METABOLITES OF ICI 118,630

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Very low doses, combined with structural features renders some of the traditional methods of metabolite identification of little use for peptide drugs. For ICI 118,630, the presence of parent compound in urine was confirmed by bioassay. Tandem MS was used successfully to identify the metabolites in urine and serum from rat and dog. Urine and serum samples were fractionated by reverse phase HPLC prior to analysis by tandem MS. Comparison of the spectra from extracts and standards confirmed the identity of a range of compounds, ICI 118,630 itself and a number of metabolites including 4-10, 5-10, 5-9, 5-7, 1-8 and 1-7. An additional metabolite, present in urine was confirmed as 5-8 on the basis of its daughter ion fragmentation pattern. Subsequent identification, in other species where the supply of material is even more limited has been based on co-chromatography in two HPLC systems.

GENETICALLY ENGINEERED V79 CHINESE HAMSTER CELLS FOR STABLE EXPRESSION OF HUMAN CYTOCHROME P450IIE1

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Succeeding in the genetical engineering of some V79 Chinese hamster cell lines expressing rat cytochromes P450, we aim to establish a V79 cell line for stable expression of human cytochrome P450IIE1. The ethanol-inducible cytochrome P450IIE1 is known to play an important role in the metabolic activation of nitrosamines and chlorinated hydrocarbons.

Cyt. P450IIE1 cDNA, kindly provided by F.Gonzales (National Cancer Institute, Bethesda, Maryland, USA), was cloned into a pSV-gpt derived recombinant eucaryotic expression vector and transfected into V79 cells. To verify the chromosomal integration of the cDNA and the effective expression, the clones were characterized by Southern-, Northern- and Western-blotting.

V79 cells stably expressing Cyt. P450IIE1 will be used for studying the P450-dependent oxidation of ethanol and acetone. In addition mutagenicity tests with nitrosamines (like N-nitrosodimethylamine and N-nitroso-N-methylaniline) and with several chlorinated hydrocarbons (like chloroform and carbon tetrachloride) will be carried out.

MORPHINE KINETICS AFTER DIAMORPHINE INFUSION IN PREMATURE NEONATES

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The pharmacokinetics of morphine were studied in 26 newborn premature neonates (26 - 38 weeks gestational age) who were given a loading dose of 50 $\mu g.kg^{-1}$ of diamorphine followed by an intravenous infusion of 15 $\mu g.kg^{-1}h^{-1}$ of diamorphine. Plasma concentrations of morphine were measured during the infusion at steady-state and for 24 hours after the cessation of the diamorphine infusion.

The mean steady-state plasma morphine concentration (± s.d) for a diamorphine infusion rate of 15 $\mu g.kg^{-1}h^{-1}$ was 62.5 ± 22.8 $ng.ml^{-1}$. Morphine clearance was 3.6 ± 0.9 $ml.min^{-1}kg^{-1}$, the elimination half-life was 8.9 ± 3.3 hours and the volume of distribution was 2.7 ± 1.0 $l.kg^{-1}$. Morphine elimination kinetics were described by a mono-exponential function. There was a direct relationship between the gestational age of the patients and the clearance (r^2 = 0.31; p = 0.003) and half-life (r^2 = 0.35; p = 0.01) of morphine, but no relationship was found between gestational age and volume of distribution.

Small but significant falls ($p<0.03$) were noted in blood pressure (at 30 minutes) and heart rate (at 30 minutes, 6 and 12 hours) after administration of diamorphine but these did not appear to cause any clinical deterioration and were thought to be related to the sedative effect of the drug. A significant fall ($p<0.01$) in respiration rate at 30 and 60 minutes reflected the desired intention to encourage synchronisation of the infants' breathing with mechanical ventilators.

The results suggest that the currently used dosing regimen of diamorphine achieves a safe and effective morphine concentration in the premature newborn but that the loading dose could be modified to achieve a more rapid onset of analgesia.

EXPRESSION OF DRUG METABOLIZING ENZYMES IN AN IMMORTALIZED RAT HEPATOCYTE CELL LINE

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In order to establish a stable cell line, rat hepatocytes were prepared using EDTA as a dissociating agent. This method provided cells whose metabolic capacities were preserved for a longer time period, when compared to rat hepatocytes isolated with collagenase (1).

Immortalization was perfomed by infection of hepatocytes with SV-40 virus in conditions which allowed the maintenance of a differenciated status of the cells.

Among the cell lines obtained, one of them, SV-Hep B4 was characterized. It exhibited a high differenciated profile with a secretion of specific hepatic proteins, and showed a very low activity of gamma-glutamyltransférase (5n mol.min^{-1}. mg^{-1} protein). Moreover, the expression of phase I and phase II enzymes was maintained at a stable level, even after 50 subcultures.

The comparison of the metabolic capacities of SV Hep B4 with those of freshly isolated hepatocytes and of differenciated hepatoma cell lines pointed out its usefulness as a competent in vitro biotransformation model.

1- J. Bayad, N. Sabolovic, D. Bagrel, J. Magdalou, G. Siest
J. Pharmacol. Methods. 1991 in.press.

THE INFLUENCE OF THE AQUEOUS SOLUTION CHEMISTRY OF SOME CISPLATIN ANALOGUES ON THEIR IN VITRO CYTOTOXIC ACTIVITIES

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The inorganic, transtiton metal complex cisplatin is one of the most effective chemotherapeutic agents in the cancer wards today. The aqueous solution chemistry has long been recognized as important for the cytotoxic activity of the compound. The relatively inert cisplatin molecule is considered a prodrug, requiring a chemical activation step to unmask the antitumor activity. Activation occurs when the Pt-Cl-bonds are hydrolyzed in the cell cytosol, where it is believed the highly electrophilic monoaquamonochloro- and diaqua-Pt species accumulate. These hydrolysis products react readily with bionucleophiles, amongst others the presumed target molecule DNA. Given the central role that Pt-Cl hydrolysis reactions play in the cytotoxic activity of cisplatin, an understanding of the aqueous solution chemistry of new analogues should be an important aspect of rational drug design. In this poster we report the syntheses, characterizations and in vitro cytotoxic testing of a series of mixed-amine cisplatin analogues. With the aid of a recently developed reversed phase-HPLC assay, the aqueous chemistry of the new compounds was explored. Possible correlations between the time-dependent concentrations of the various Pt-hydrolysis products and the cytotoxic potencies of the Pt-complexes in three human cancer cell lines have been investigated.

TOLBUTAMIDE AND PHENYTOIN METABOLISM BY HUMAN CYTOCHROMES P450

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The human P450 2C subfamily is a microheterogeneous group of cytochromes P450 involved in the methylhydroxylation of tolbutamide and the stereoselective hydroxylation of S-mephenytoin. In vivo, tolbutamide hydroxylation is not linked to the genetic polymorphism of S-mephenytoin hydroxylation. A method based on urinary metabolic ratio for screening tolbutamide hydroxylation in vivo has been developed. One slow metaboliser has so far been identified from approximately 80 subjects studied. In vitro with human liver microsomes there was a high degree of correlation between tolbutamide and phenytoin hydroxylations ($r=0.85$, $p<0.001$). In vivo also there was a significant correlation between AUCs for tolbutamide and phenytoin ($r=0.87$, $p<0.01$). A human cytochrome P4502C9 cDNA was isolated from a human liver lambda gt11 library and expressed in COS cells. The expressed enzyme metabolised tolbutamide with a Km similar to that with human liver microsomes and catalyzed the 4-hydroxylation of phenytoin. The selective inhibitor sulphapenazole inhibited both activities with a potency similar to that with human liver microsomes. Phenytoin competitively inhibited tolbutamide hydroxylation with a Ki similar to the Km for phenytoin hydroxylation by microsomes. The results suggest that phenytoin and tolbutamide are hydroxylated by (a) similar isozyme(s). Site directed mutagenicity studies are in progress to investigate the effect of microheterogeneity in the 2C9 subfamily on catalytic function towards tolbutamide, phenytoin and mephenytoin.

SPECIES DIFFERENCES IN DEACETYLATION RATES OF MERCAPTURIC ACIDS OF SEVERAL HALOALKENES

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Measuring the excretion of mercapturic acids (MA) in urine is proposed to be a tool to quantitate the formation of electrophiles in the metabolism of haloalkenes. Glutathione S-conjugates formed from nephrotoxic haloalkenes are further processed to cysteine S-conjugates in the kidney. These conjugates are the precursors for toxic metabolites formed by ß-lyase as well as for the excreted mercapturic acids. Determination of the concentration of MA's should permit conclusions about the concentration of formed toxic metabolites. For this purpose, an exact knowledge of the enzyme activities catalyzing these pathways is necessary. We compared the rates of deacetylation of several MA's in Wistar and Fischer 344 rats, since deacetylation is considered to be the decisive step for toxic effects in the kidney. Species differences in the deacetylation rates [nmol/mg*min] were found: *N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine*: Wistar: 0.3 (kidney), 0.06 (liver); Fischer 344: 0.6 (kidney), 0.2 (liver); *N-acetyl-S-(2,2-dichlorovinyl)-L-cysteine*: Wistar: 0.4 (kidney), 0.07 (liver); Fischer 344: 0.6 (kidney), 0.2 (liver). *N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine*: Wistar: 0.1 (kidney); Fischer 344: 0.2 (kidney). Homogenates of rat kidney, which is the target organ for the toxicity of haloalkenes metabolized by S-conjugate formation, show the highest activity for deacetylation of these MA's. Species differencies between Wistar and Fischer 344 rats may also contribute to observed differences in susceptibility to nephrotoxic effects. However, the influence of other parameters as ß-lyase activity or uptake has to be investigated.

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HUMAN C-S LYASE ENZYMES: TRANSAMINATION AND STRUCTURE-ACTIVITY RELATIONSHIPS

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Cysteine conjugate β-lyase (C-S lyase, EC 4.4.1.13) from rat renal tissue has been identified as a transaminase: glutamine transaminase K (GTK). We have examined partially purified C-S lyase enzymes from renal, hepatic, and pulmonary tissue for their transamination activity towards L-phenylalanine (GTK), L-albizziin (glutamine transaminase L, GTL), and L-kynurenine (kynurenine aminotransferase, KAT). C-S lyase activity, in the presence of pyridoxal phosphate, was determined by assaying for the pyruvic acid formed as a product of the C-S lysis of S-(*E*-1,2-dichlorovinyl)-L-cysteine (DCVC). The C-S lyase activity of the isolated enzymes for the following conjugates was also examined: S-(1,1,2-trichlorovinyl)-, S-(2-chloro-1,1,2-trifluoroethyl)-, S-(2-chloro-1,1-difluoroethyl)-, S-benzothiazolyl-, S-phenyl-, and S-(4-bromophenyl)-L-cysteine.
Structure-activity relationship studies on each of these substrates showed that the cytosolic enzyme fractions were broadly similar in terms of their specific activities as those determined from mitochondrial fractions. The substituted aromatic compounds were generally poorer substrates when compared to those conjugates derived from halogenoalkenes. Pulmonary cytosolic C-S lyase specific activity (nmol/min/mg) was much less (0.22) than those of renal (3.63) and hepatic (3.67) origin. GTK activity was determined at a higher level than GTL activity in both renal and hepatic tissues. KAT activity was found at a significant level in renal, pulmonary, and hepatic cytosolic fractions.
This work was generously supported by the Health and Safety Executive (studentship to LDB).

EVIDENCE FOR OXIDATIVE ACTIVATION OF MITOXANTRONE IN MAN, PIG AND RAT

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Intercalation and electrostatic interaction with the DNA are considered to be the main reasons for the cytotoxicity of the anticancer agent mitoxantrone. A new metabolite, less polar than mitoxantrone, was now detected in human, rat and pig urine and characterized by tandem mass spectrometry and UV-VIS spectroscopy as 8,11-dihydroxy-4-(2-hydroxyethyl)-6-[[2-[(2-hydroxyethyl)amino] ethyl]amino]-1,2,3,4,7,12-hexahydronaphtho-[2,3-f]-chinoxaline-7,12-dione. The cytotoxic activity of the metabolite was determined using an *in vitro* clonogenic assay with human xenocrafts. Within the concentration range tested (0.01-3 µg/ml), the metabolite showed significant reduced cytotoxicity compared with mitoxantrone.

The metabolite was synthesized from mitoxantrone by enzymatically catalyzed reaction under the formation of a highly reactive quinone-diimine. The alkylating property of this intermediate could be demonstrated by reaction with glutathione. The structures of the conjugation products have been elucidated by means of tandem mass spectrometry with ion spray as ionisation method and ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy. The formation of glutathione conjugates was already observed in vitro during incubation of human and rat liver microsomes (Wolf et al.) but no structural information was reported. The occurrence of this metabolite proves oxidative activation of the drug and implies that alkylation may be a further way of action.

CIMETIDINE / ASCORBIC ACID AND CYTOCHROME P 450

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Cimetidine is a drug including a free imidazole ring. Imidazoles have proven to react with cytochrome p 450. With etomidate it is proven that the cortisol synthesis is blocked in this way. Ascorbic acid can restore cortisol synthesis. Cimetidine is an imidazole which reacts with cytochrome p 450 and has not been tested clinically.

One study has been performed in ten, male volunteers, during major abdominal vascular surgery. The effect of cimetidine on steroidogenesis is compared, in a double blind prospective study, with the effect of ranitidine. A reduction of 40 percent has been measured in the group of patients treated with cimetidine. In the ranitidine group it is not possible to demonstrate significant differences in serum cortisol concentration.

Another study is a blinded parallel, prospective, clinical study. It whas been conducted to investigate the effect of ascorbic acid on human serum cortisol concentrations which are decreased by the administration of cimetidine. The study population included 16 male adults scheduled for major abdominal vascular surgery. The results show a significant reduction in serum cortisol concentrations in patients receiving a placebo. Patients receiving ascorbic acid have a significant increase in serum cortisol.

Conclusion: ascorbic acid can prevent cimetidine induced decrease of human serum cortisol.

THE EFFECTS OF FOOD ON THE BIOAVAILABILITY AND THE DISPOSITION OF SINGLE ORAL DOSES OF PIROXIMONE, A NEW CARDIOTONIC AGENT

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Piroximone (MDL 19205), a 4-aroylimidazole-2-one is a specific inhibitor of a high affinity cAMP phosphodiesterase (phosphodiesterase type III) and is currently developed for the treatment of congestive heart failure. Pharmacokinetic (PK) parameters were studied in response to intravenous and oral administration of single doses of 25 and 50 mg of piroximone in 2 separate groups of n = 6 healthy male volunteers. In addition, PK parameters were assessed under fasting conditions and compared to PK parameters obtained after the consumption of a standard breakfast. The study was conducted as a 3-way crossover randomised design. PK parameters were not affected by food administration, i.e. an overall mean $t_{\frac{1}{2}}$ of 1.5 ± 0.3 h (mean ± SD, n = 36) was determined. No significant difference was found in $t_{\frac{1}{2}}$ calculated from the decay of plasma concentration or from urinary excretion. Intravenous and oral administration of 25 mg of piroximone resulted in AUC values ($nmol.h.ml^{-1}$) of 2.73 ± 1.10 (i.v.) and 2.19 (p.o.) whereas 50 mg administration yielded AUC values of 5.59 ± 1.07 (i.v.) and 4.11 ± 0.78 (p.o.), respectively. AUC values were not altered under fasting conditions or with the consumption of a standard breakfast. The bioavailability calculated from AUC data ranged from 88 to 95 % for a single dose of 25 mg and 74 to 76 % for a single dose of 50 mg piroximone. Maximal plasma concentrations (t_{max}) were observed between 0.5 to 0.9 h.

COMPARISON OF THE METABOLIC PROFILES OF ^{14}C-ANETHOLE IN THE RAT AND ISOLATED RAT HEPATOCYTES

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trans- Anethole is an important anisole derivative flavour compound. It occurs naturally in the volatile oils of a variety of plants and is used widely in aniseed flavoured baked goods, sweets and beverages.

Previous *in vivo* studies (1) using [*methoxy*-^{14}C]-anethole have indicated that its metabolism proceeds along three major pathways : O- demethylation (as indicated by the exhalation of $^{14}CO_2$), ω-oxidation and epoxidation of the side chain double bond. The abundance of metabolites from each pathway along with the excretion kinetics are markedly affected by the administered dose size , sex and species.

By employing radio-HPLC we have been able to compare rat urine profiles from animals dosed with either [*methoxy*-^{14}C] or [*side chain*-^{14}C]-anethole. This now reveals that the O-demethylated moiety undergoes further metabolism, and the structures of these new metabolites are currently being elucidated.

The radio-HPLC profiles of rat liver hepatocytes dosed with [*side chain*-^{14}C] - anethole show only one major route of metabolism. This is via ω-oxidation of the side chain, yielding 4-methoxycinnamyl alcohol (a putative metabolite from the *in vivo* studies). This is then oxidized further to 4-methoxycinnamic acid, which in turn undergoes β-oxidation to 4-methoxybenzoic acid. The hepatocytes do not appear to conjugate this with glycine, so that 4-methoxyhippuric acid - the major *in vivo* metabolite - is not seen. The extent of metabolism by the other two pathways, epoxidation and O-demethylation, is very minor in the isolated hepatocytes compared to the *in vivo* studies.

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GLUTATHIONE-RELATED METABOLISM OF FOTEMUSTINE IN THE RAT: URINARY EXCRETION OF SULFUR-CONTAINING METABOLITES.

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Fotemustine is a new chemotherapeutic drug, used in the treatment of malignant melanoma. Both phase III clinical trials and clinical studies have shown that fotemustine causes far less general toxicity than classical nitrosourea. In contrast to nitrosourea such as BCNU, fotemustine seems devoid of inhibiting properties on several rat-tissue derived glutathione reductases. In as much as glutathione (GSH)-related metabolism is hypothized to affect the therapeutic effect of fotemustine (through inactivation and detoxification), GSH-related metabolism of the 2-chloroethyl-N-nitrosourea moiety of fotemustine was investigated. The compound was hypothesized to be metabolized in one or more cytostatically active intermediates, which would be inactivated by further metabolism under formation of at least six acidic GSH-derived metabolites (thiodiacetic acid (TDA), N-acetyl-L-cysteine (NAC), methyl N-acetyl-L-cysteine, carboxymethyl-L-cysteine, carboxymethyl N-acetyl-L-cysteine and 2-hydroxyethyl N-acetyl-L-cysteine). The sum of these metabolites would be an indication for inactivation of the antitumor activity of fotemustine. Thus, these metabolites were synthesized, identified with NMR and mass spectrometry and, subsequently, gas chromatographic methods based on capillary GC with sulfur (GC-FPD) and mass spectrometric (GC-MS) detection were developed for their identification and quantification in urine. Following treatment of rats with fotemustine (40mg/kg i.p.), TDA and NAC could be identified in urine. In 24 and 48 hours urine these metabolites were found to be excreted for 15 ± 10% (± 2, n=3) and about 1% (n=4) of the dose in total, respectively. No detectable amounts of the other sulfur containing metabolites were present in urine (meaning less than 0.1% of the dose was excreted in 48 hours urine). The formation of sulfoxides and sulfones from these thioether metabolites is presently under investigation.

EFFECT OF MULTIPLE RIFABUTIN ADMINISTRATION ON ISONIAZID PHARMACOKINETICS AND METABOLISM IN HEALTHY VOLUNTEERS

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Rifabutin (RIF) was frequently used in combination with isoniazid (I) in clinical trials for the treatment of tuberculosis. The aim of this study was to assess whether repeated administration of therapeutic doses of RIF affects the pharmacokinetics and metabolism of I. Six healthy male volunteers received on day 1 and 9 of the investigation a single oral dose of 300 mg I. Blood samples were collected up to 24 h after drug administration. From day 2 to 8 each subject received a single daily dose of 300 mg RIF p.o. Blood samples were collected 24 h after the first, the fifth, the sixth and the seventh RIF administration. Plasma concentrations of I, acetylisoniazid (AI), RIF and 25-O-deacetyl RIF were measured by two different HPLC methods. Treatments were well tolerated. Two out of the six subjects were shown to be rapid acetylators. Their mean pharmacokinetic parameters were: Cmax 5.13 and 5.24 μg/ml, $AUC_{0-\infty}$ 9.64 and 47.74 μg.h/ml, MRT 2.28 and 7.13 h, $t_{\frac{1}{2}}$ 1.36 and 4.20 h for I and AI, respectively. The corresponding mean values in slow acetylators were 6.76 and 2.21 μg/ml, 23.16 and 29.59 μg.h/ml, 3.78 and 9.12 h, 2.51 and 5.05 h. No significant modification of the plasma pharmacokinetic parameters of both I and AI, following the treatment with RIF, was found. As shown in a previous study, autoinduction of RIF metabolism appears to have occurred also in the present investigation.

CYTOCHROME P-450s EXPRESSION IN RAT PLEURAL MESOTHELIAL CELLS IN SECONDARY CULTURES

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The presence of cytochrome P-450 in rat pleural mesothelial cells (RPMC) has been suggested by the previous demonstration in our laboratory showing the metabolism of benzo(a)pyrene to dihydrodiols in secondary cultures.The aim of this study was to characterize the expression of several isozymes of cytochrome P-450 in mesothelial cells from male Sprague-Dawley rats during secondary cultures. Cells cultured in Ham's F10 medium supplemented with 10% fetal calf serum (In Vitro 17:98-106, 1981) were studied between passages 9 to 16 for apoprotein and mRNA expression. Several cytochrome P-450 inducers were added to the medium of subconfluent cultures for 24 to 48 hours: 0.1 μM dexamethasone, 10 μM 3-methylcholanthrene, 170 mM ethanol or 2 mM phenobarbital.

Western blot analysis of microsomal proteins of untreated cells allowed detection of cytochrome P-450 isoforms IA1, IA2, IIE1, and several forms recognized by polyclonal antibodies against isoforms IIC11 and IIIA1. Isoforms IIB1 and IIC12 were not detected. This expression pattern was found in all cell strains issued from different rats. Cytochrome P-450IA1 and P-450IIE1 apoproteins were induced by 3-methylcholanthrene and ethanol respectively. Cytochrome P-450IA1 mRNA level (Northern blot) was markedly increased by 3-methylcholanthrene treatment, whereas ethanol had no effect on cytochrome P-450IIE1 mRNA. Dexamethasone had no clear effect on cytochrome P-450IIIA family but was a strong inducer of the cytochrome P-450IIE1 mRNA, despite of a slight effect on cytochrome P-450IIE1 protein expression.

In situ hybridization with mRNA probe specific for P-450IA1 showed that only a part of the cells were strongly labelled in the presence of 3-methylcholanthrene. This heterogeneous response was not explained by cytotoxicity of 3-methylcholanthrene metabolites (MTT test and trypan blue exclusion) and might indicate an evolution of cell susceptibility to inducer according to factors such as cell cycle or age.

Detection of epoxide hydrolase (Western blot) in RPMC in addition to cytochrome P-450IA1 demonstrates the presence of phase I and phase II enzymes allowing formation of mutagenic metabolites of polycyclic aromatic hydrocarbons.

Maintenance of morphologic and enzymatic differentiation in secondary culture of RPMC might be related to the ability of these cells to synthesize an abundant extracellular matrix. Rat pleural mesothelial cells may constitute an interesting model, complementary to hepatocytes, for the in vitro alternative studies on xenobiotic metabolism and cytochrome P-450 regulation.

DEPRESSION OF CYT P450 BY INFLAMMATORY MEDIATORS

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Murine rec TNF (20,000 or 40,000 units) produced in vivo depression of hepatic cytochrome P450 at 12 and 24 hours, but not at 6 hours post-injection. Cytochrome P450 content and EROD activity was depressed by about 30%. TNF is a known inducer of the hepatic "acute phase response" to inflammation, therefore the effect on albumin expression (a negative acute phase reactant) was examined via Northern analysis. A depression of albumin mRNA 24-hours post-TNF injection suggested that an acute phase response was effected in parallel with cytochrome P450 depression. TNF induces an acute phase response in part through IL-6, therefore the role of IL-6 in TNF-mediated depression of P450 was assessed both in vitro and in vivo. A 10% depression of hepatic cytochrome P450 content was seen in experiments where mice were treated in vivo with rec murine IL-6. When primary cultures of hepatocytes were incubated with rec murine IL-6, a dose-dependent depression (up to 50%) of both cytochrome P450 content and aminopyrine N-demethylase activity occurred. These results suggest that the inflammatory depression of cytochrome P450 is effected in part by known mediators of the hepatic acute phase response.

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PURIFICATION AND CHARACTERISATION OF HUMAN HEPATIC C-S LYASE

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The C-S lyase enzymes are responsible for the generation of mutagenic and cytotoxic metabolites *via* aberrant drug metabolizing pathways in mammalian tissues. We have examined human hepatic cytosolic and mitochondrial fractions for evidence of C-S lyase activity. S-(*E*-1,2-Dichloroethenyl)-L-cysteine was used as the substrate (10mM) and enzyme activity was determined by assaying for pyruvic acid. C-S lyase specific activity (nmol/min/mg) in cytosol was 3-fold greater than that observed in mitochondria (2.55±0.20 vs. 0.85±0.30) and enzyme activity did not increase on the addition of sodium α-keto-γ-methiol-butyrate. Co-incubation with pyridoxal phosphate resulted in a marked increase in the mitochondrial specific activity (3.15±0.20), but in only a minor improvement in that of the cytosolic enzyme (3.20±0.30). The optimum pH for enzyme activity, in both fractions, was 7.5.
The cytosolic enzyme was purified using FPLC over FFQ Sepharose, Mono P (chromatofocusing), and Superose 12. The transaminase activity of the enzyme with three substrates: kynurenine, phenylalanine, and albizziin (an analogue of glutamine) was examined. A homogeneous protein (monitored by SDS-PAGE) was obtained following purification, and a 15-fold increase in specific activity was observed. The molecular weight of the enzyme was found to be 37kDa in denaturing conditions, 82.3kDa in non-denaturing conditions, and was shown to co-purify with kynurenine aminotransferase.

We acknowledge the financial support of the Health and Safety Executive (studentship to LDB).

HUMAN RENAL C-S LYASES: TWO CYTOSOLIC ISOENZYMES

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A deviation from the glutathione conjugation pathway involves the C-S lyase (CSL) enzyme which catalyzes the cleavage of a cysteine conjugate to yield a potentially toxic product. Human renal tissue was obtained *post mortem* and cytosolic and mitochondrial fractions wereprepared. The cortical cytosolic fraction was purified by Fast Protein Liquid Chromatography. CSL activity was determined by assaying for the pyruvic acid formed as a product of the C-S lysis of S-(*E*-1,2-dichlorovinyl)-L-cysteine (DCVC) and of S-benzothiazolyl-L-cysteine (BTC). Glutamine transaminase K (GTK) activity was measured by monitoring the transamination of L-phenylalanine in the presence of sodium α-keto-γ-methiolbutyrate (KMB).
The CSL specific activities (nmol/min/mg), using DCVC as the substrate, were observed to be greatest in cortical microsomes (6.07±0.57), followed by medullary microsomes (5.25±1.75). The values for CSL activity in the cytosol and mitochondria were generally significantly smaller than these values. BTC was a poorer substrate for CSL activity than DCVC. Two essentially homogeneous (SDS-PAGE) proteins which displayed both CSL and GTK activity were purified. The two proteins had different relative molecular weights, but had the same isoelectric point. Exogenous pyridoxal phosphate was not required for CSL activity, but KMB was essential to maintain activity during the enzyme purification.
This work was generously supported by the Health and Safety Executive (studentship to LDB).

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CHEMICAL AND XENOBIOCHEMICAL STUDIES ON AFLATOXINS AND AFLATOXIN MODELS

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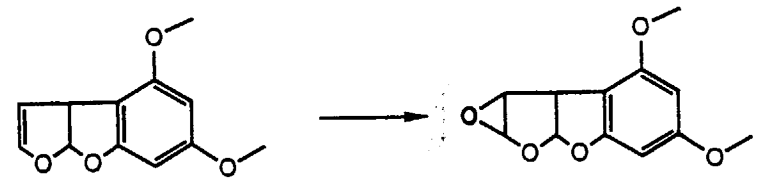
The synthesis and mutagenicity (Salmonella/Ames test) of the aflatoxin model 1 is presented. Data on the mutagenicity of AFB_1 8,9-epoxide will be also shown for comparison purposes. Furobenzofuran 1 exhibited a moderate direct mutagenicity in TA-100 strain, which was enhanced in the presence of S9 fraction. Its epoxy derivative 2 showed a similar activity pattern. However, the fact that indirect mutagenicity elicited by 1 was greater than direct mutagenicity exhibited by oxirane 2 suggests that bioactivations other than epoxide formation could contribute to the overall cytotoxicity observed for compound 1. In this context, the extent at which furobenzofuran 1 could be taken as model for studying bioactivation mechanisms alternate to epoxide formation in aflatoxins will be discussed.



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On the other hand, preliminary results on the preparation of aflatoxicol 8,9-epoxide and on the reactivity of this intermediate and AFB_1 8,9-epoxide with selected nucleophiles will be also presented.

EVIDENCE FROM DWARF RATS THAT GROWTH HORMONE MAY NOT REGULATE THE SEXUAL DIFFERENTIATION OF LIVER P-450 ENZYMES AND STEROID 5α-REDUCTASE.

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Differences in the pattern of growth hormone secretion in mature rats (i.e., "continuous" secretion in females *versus* "pulsatile" secretion in males) are thought to be the underlying cause of sex-dependent differences in a subpopulation of liver microsomal P450 enzymes and steroid 5α-reductase. A new strain of dwarf rats (NIMR/AS) has recently been shown to have low or undetectable levels of circulating growth hormone due to a selective defect in pituitary growth hormone synthesis. We have measured the levels and/or activity of IIA1 (P450a), IIA2 (P450m), IIC11 (P450h), IIC12 (P450i), IIIA2 (a P450p isozyme) and steroid 5α-reductase in liver microsomes from male and female dwarf rats, to test the hypothesis that the expression of these sexually dimorphic enzymes is regulated by growth hormone. In mature rats, the levels of liver microsomal IIA2, IIC11 and IIIA2 were higher in male than in female dwarf rats, whereas the levels or activity of IIA1, IIC12 and steroid 5α-reductase were greater in female than in male dwarf rats. These sex differences resulted from age-related changes in either male dwarf rats (i.e., an increase in IIC11 and IIA2, and a decrease in IIA1) or female dwarf rats (i.e., an increase in IIC12 and 5α-reductase, and a decrease in IIIA2). The magnitude of these sex-dependent, age-related changes was essentially indistinguishable from those observed in normal rats. These results were unexpected, and raise the possibility that growth hormone is not the pituitary factor responsible for regulating the levels of sexually dimorphic, steroid-metabolizing enzymes in rat liver. Alternatively, it is possible that these enzymes are regulated by extremely low levels of growth hormone. In either case, the current model of how steroid-metabolizing enzymes are regulated in rats must be revised to account for the normal sexual differentiation of these enzymes in dwarf rats.

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REGULATION OF CYTOCHROME P450IVA1 GENE EXPRESSION BY AN INTERFERON MEDIATED MECHANISM

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Interferons have been shown to depress the activity of the cytochrome P450 system. This effect has profound implications on the biotransformation of drugs used in conjunction with interferons in the treatment of a variety of disorders.

Our studies to date have suggested that interferons suppress drug metabolism by causing a decrease in the synthesis of cytochrome P450 apoprotein, thereby reducing total P450 protein content and activity. In particular we have demonstrated the inhibitory effect of an interferon inducer, poly IC, on the level of P450 specific content and on the content and activity of the clofibrate-induced isozyme P450IVA1. Furthermore, the level of clofibrate-induced P450IVA1 mRNA is also reduced in poly IC-treated rats. This result suggests that the P450IVA1 gene may be down-regulated by an interferon mediated mechanism, which may be at the level of gene transcription or mRNA destabilisation. The former possibility is currently being investigated by nuclear run-on experiments.

EVIDENCE FOR TANGERETIN DEMETHYLATION BY RAT LIVER MICROSOMES

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Tangeretin, a naturally occurring polymethoxylated flavonoid, has been shown to induce drug metabolizing enzymes in the rat. Thus, it may be considered as a potential anticarcinogenic compound. However, there is no information about its metabolism. In the present study, it was shown that *in vitro* incubation of tangeretin with rat liver microsomes produced formaldehyde. The demethylation required NADPH and O_2. Classical inhibitors of cytochrome P-450 lowered the production of formaldehyde. In addition, piperonyl butoxide, 7,8-benzoflavone and 5,6-benzoflavone strongly inhibited the reaction. Microsomes extracted from rats treated with methylcholanthrene metabolized tangeretin to a much greater extent than did microsomes from rats treated with phenobarbital, dexamethasone, or clofibrate. Incubation of tangeretin with microsomes gave rise to a reverse type I spectrum.
All these observations suggested that tangeretin is likely to be demethylated by a cytochrome P450 IA- dependent monooxygenase.

EXPRESSION OF OXIDATIVE AND POSTOXIDATIVE ENZYMES IN NOVEL MURINE LIVER EPITHELIAL CELL LINES AND THEIR ABILITY TO ACTIVATE PROCARCINOGENS.

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Four novel nontransformed epithelial cell lines, isolated from fetal or adult mouse liver, were tested: (a) to determine the profile of metabolizing enzymes; (b) to evaluate the inducibility of the monooxygenase system; and (c) to assess the capacity to metabolize structurally different procarcinogens. The cells expressed class IA, IA2, IIB, IIE1, IIIA-P450s-and FAD-containing monooxygenase -dependent enzyme activities at levels (in lines C2.8 and C6) comparable with those present in murine adult liver. For the phase II pathway, cells expressed substantial levels of glutathione S-epoxide- and S-transferase, and UDP- glucuronosyltransferase. Low expression of epoxide hydrolase was observed. Induction of P450 function by phenobarbital, β-naphthoflavone, isosafrole, ethanol, and pregnenolone 16α-carbonitrile, was considerably elevated (over 5-fold in class IIB with the C2.8 and C6 lines). The most competent C2.8 and C6 lines were able to activate benzo(a)pyrene (BaP), cyclophosphamide (CP), dimethylnitrosamine (DMNA), diethylstilbestrol (DST), and 2-naphthylamine (2NPA) in S.cerevisiae D7 after 4(CP), 24(BaP, 2NPA, DMNA) or 48(DST) h of exposure with 3 x 10^6 cell/flask. The degree of conservation and the inducibility of representative metabolizing reactions in C2.8 and C6 lines, together with their ability to activate procarcinogens, offers a means to study the potential of chemicals for inducing DNA damage, for analyzing the metabolic disposition of compounds and the multistage process of carcinogenesis.

INHIBITION OF LIPID PEROXIDATION IN RAT LIVER MICROSOMES BY SIMPLE 2,2-DIMETHYLCHROMANS AND CHROMENES

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The effects on lipid peroxidation induced by a series of simple 2,2-dimethylchromans and chromenes with substituents at the C-6 and/or C-7 position, using rat liver microsomes as biological matrix, and evaluated by the MDA/thiobarbituric assay and the oxygen consumption rate test, are presented.

All the compounds assayed showed an inhibitory activity of lipid peroxidation although the effect obtained was dependent on the nature of the aromatic ring substituents. Among the compounds tested, a hydroxychroman derivative with a OH group at C-6 exhibited the highest inhibition effect, with a IC_{50} = 0.3 μM (MDA test). This value was in the same order of magnitude than that obtained for BHT.

The data collected from the compounds tested so far suggest that the antioxidant effect found is a consequence of a vitamin E-like mechanism and it depends from a combination of several factors. Thus, the presence of a free OH group at C-6 or better, its putative formation by a cytochrome P-450 mediated bioactivation process, and the introduction of groups that could prevent the monooxygenase metabolism at other activated positions of the molecule, could be properly combined for obtaining relatively simple lipophilic compounds which could exert a selective antioxidant activity. These compounds would act as prodrugs by giving rise to the free radical scavenger species in the environment where these cytotoxic intermediates are mostly generated. Preliminary results obtained on this approach will be also discussed.

INFLUENCE OF MODULATORS OF SULFATION ON THE GENOTOXICITY OF METHYLEUGENOL (ME) AND 1'-HYDROXYMETHYLEUGENOL (HME) IN RAT HEPATOCYTES

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The genotoxicity of carcinogenic allylbenzenes such as ME is seen in the Unscheduled DNA Synthesis (UDS) assay in rat hepatocytes but not in bacterial tests (1) owing to the difficulty of assuring their metabolic activation in the Ames' test. Their activation involves 1'-hydroxylation of the side chain followed by sulfation. We have shown the enhanced genotoxicity in the UDS system of the 1'-hydroxy metabolites over the parent allylbenzenes (2) and now report on the influence of modulation of sulfation on the UDS response to ME and HME, studied as previously described (1,2).

The 3'-phosphoadenosine-5'-phosphosulfate (PAPS) precursor inorganic sulfate, enhanced the UDS response, up to 205%±29% (x±SE, n=3) of control with ME and 127.5%±7% with HME at 5mM Na_2SO_4. L-cysteine, but not its D-antipode, increased the UDS response to HME up to 201%±9% of control. No treatment influenced cytotoxicity. Inhibition of sulfation with pentachlorophenol (PCP) decreased the UDS response in a dose-related fashion, with complete inhibition, decreased to 0.2%±0.2% of control to ME and 1.4%±1.4% to HME, seen at 5 and 10μM PCP respectively. Inhibition of glutathione synthesis with buthionine sulfoximine (BSO) enhanced the cytotoxicity of HME but reduced the UDS response up to 45%±14% at non-cytotoxic concentrations. This paradoxical response, glutathione generally being associated with cytoprotection against electrophilic intermediates is accounted for by the dose-related reduction to 24% of control of intracellular PAPS levels caused by 0.1mM BSO.

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CYTOCHROME P450IVA CHARACTERISATION IN HUMAN LIVER MICROSOMES.

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Human liver tissue from both males and females aged between 35-45 years have been investigated for the expression of the cytochrome P450IVA subfamily and a structural comparison has been made with a similar form expressed in the rat (P450IVA1).

All the microsomal preparations showed cross-reactivity with sheep anti-sera to cytochrome P450IVA1 purified from clofibrate-induced rat and gave a single band on Western blots with a molecular weight of approximately 51 kDa on a 10% SDS-PAGE gel. Two bands have previously been reported with rat microsomes and the lower more intense one of approximately 51.5kDa corresponded to cytochrome P450IVA1 isozyme. Spectral analysis of ferrous-carbon monoxide complexes revealed peak maxima between 450-452 nm and total cytochrome P450 specific content of 0.2-0.6 nmol./mg microsomal protein as well as cytochrome P450 reductase activity between 18-37 nmol./min./mg microsomal protein. Immunoquantitation of the cytochrome P450IVA1 by an adapted ELISA protocol gave between 4-12 % of total cytochrome P450. NADPH-fortified human liver microsomal preparations showed mainly the 12-hydroxylase activity towards lauric acid as substrate, this activity being of the same order of magnitude as rat liver microsomes.

Taken collectively, the information thus presented is strongly suggestive of the expression of a member(s) of the cytochrome P450IVA subfamily in the human liver tissues examined.

A POTENTIALLY NEW METABOLIC PATHWAY : ETHYL ESTERIFICATION OF ACITRETIN

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Both acitretin (Neotigason®, Soriatane®) and etretinate (Tigason®, Tegison®) are marketed for the treatment of psoriasis. Etretinate (ethyl ester) is considered a prodrug for acitretin (carboxylic acid). Recently we have found varying concentrations of etretinate (traces - 60 ng/ml) in plasma of patients treated with acitretin. However, ethyl esterification is not considered a normal metabolic pathway.

The present study investigated the formation of etretinate from acitretin in vitro (liver homogenate, 12000 g supernatant) and in vivo, with analysis by HPCL/UV and LC/MS. The formation was confirmed with both rat and human liver homogenates (37°C, 4 h). Etretinate was detected after incubation with and without ethanol addition, but not when enzymes were degraded by heating to 100°C for 10 min. Therefore, ethanol enhances, but is not essential for the formation of etretinate. Furthermore, this esterification involves enzymic catalysis. After a single oral dose of acitretin in rats, very low concentrations of etretinate (1-2 ng/ml) could be detected in plasma. This etretinate formation was significantly amplified after oral administration of ethanol, either simultaneously with acitretin or 4 to 8 hours thereafter.

BIOTRANSFORMATION OF MONO-HALOGENATED ANILINES.

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Halogenated anilines are used in industry for synthesis of pesticides, herbicides and pigments. The main purpose of our studies is to elucidate the influence of substituent pattern in the aromatic aniline-ring on the *in vitro* and *in vivo* biotransformation, with special emphasis on the bioactivation of these compounds.
Further, we want to predict the biotransformation reactions, reactivity, stability and finally the toxicity of the halogenated anilines and their metabolites with the aid of molecular orbital calculations.
In order to investigate the metabolism of fluorinated anilines *in vitro* as well *in vivo*, a ^{19}F-n.m.r. technique was developed, which enabled us to detect metabolite patterns for fluorinated anilines. The poster will present results of the *in vitro* and *in vivo* biotransformation of 2-,3- and 4 fluoroaniline obtained by using ^{19}F-n.m.r.
^{19}F-n.m.r. spectra of microsomal hydroxylation, conversion by hepatocytes and urine of exposed rats will be shown. Data will be presented that demonstrate the measurement of uptake and excretion of 2-fluoroaniline in the liver and bladder of an intact animal by *in vivo* ^{19}F-n.m.r.
Some correlations between biotransformation characteristics and outcomes of molecular orbital calculations will also be presented.

MEASUREMENT OF FLAVIN-CONTAINING MONOOXYGENASE ACTIVITY IN FRESHLY ISOLATED HEPATOCYTES AND THEIR CULTURES.

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Flavin-containing monooxygenase (FMO) is a microsomal phase I enzyme, catalysing the oxygenation of a wide range of nucleophilic N-, S-, Se- and P-containing xenobiotics. It plays therefore a key role in the biotransformation and detoxication of numerous drugs and the maintenance of its expression in "in vitro" systems, proposed as alternatives in pharmaco-toxicological research, is essential.
Several methods, measuring the enzymatic activity of FMO in biological samples have been discribed but until now, no simple, reliable and sensitive technique, applicable to isolated hepatocytes and their cultures, has been reported. In this study, a spectrophotometric method, modified from the work of Dixit and Roche and suitable for the assessment of FMO activity in the microsomes of hepatocytes, has been developed. The reproducibility is good (CV = 2.1 %, n = 12) and the sensitivity is about 0.5 nmol/min/mg protein. Linearity is obtained up to protein concentrations of 10 mg/ml. The pH-optimum lays around 8.7 when the optimized buffer concentration of 0.25 M tricine is used. Optimal concentration of the reagents are found to be 22.5 µM and 60.0 µM of dithiotreitol and 5,5'-dithio-bis-(2- nitrobenzoate), respectively. Interferences by endogenous glutathione or glutathione reductase are not observed in washed microsomes, simplifying the original method since treatment with N-ethylmaleïmide is not longer necessary. From these results it is concluded that Km is 40.6 µM and Vmax is 11.9 nmol/min/mg protein. No interferences from cytochrome P-450 dependent monooxygenases could be observed. Finally, microsomes of rodent hepatocytes can be kept for at least 30 days after freezing in liquid nitrogen, followed by storage at - 80 °C.